ECOLOGY AND THERMAL INACTIVATION OF MICROBES IN AND ON INTERPLANETARY SPACE VEHICLE COMPONENTS

Forty-fifth Quarterly Report of Progress

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THERMAL RESISTANCE OF *STAPHYLOCOCCUS AUREUS* STRAIN 472 AT 60 C AND ELEVEN MOISTURE LEVELS (% RH)

INTRODUCTION

A number of reports have dealt with the thermal resistance of vegetative cells at a_w between 0.8 and 1.0. Baird-Parker et al. (2) performed extensive reviews of *Salmonella* strains and reported a_w experiments from 0.85 to 0.998. The a_w in solution is achieved by varying concentrations of NaCl, glycerol, and sucrose. The thermal resistance of *Salmonella* increases in most cases. However, these solutions have the disadvantage that with increased concentrations of NaCl and glycerol, the organisms are adversely affected. Sucrose enhances the growth of some organisms, and therefore, may distort the measurements of thermal resistance.

The present investigation was designed to cover the % RH range of 0.19 to 100 at 60 C. A solution of given a_w should have the same RH in the headspace at equilibrium in a closed system. The advantage of studying *Staphylococcus aureus* - 472 in our system (3) is the extension of the moisture range that can be examined. Since only one temperature has been tested thus far, correction for comeup times was performed using median value (Z = 5.5 C) from the literature.

MATERIALS AND METHODS

Test organism

S. aureus strain 472 was obtained from Dr. G. K. Murthy, Food and Drug Administration, Cincinnati, Ohio.

Preparation of inoculum

Cell cultures were grown in trypticase soy agar (TSA) slants fortified with 0.2% yeast extract and 0.1% soluble starch, and were stored at room temperature. For each experiment, a loopful of inoculum from each slant was introduced into a 50-ml Falcon conical tube containing 10 ml of fortified Trypticase soy broth. This tube was thoroughly mixed by vortexing, and was incubated for about 24 h at 35 C. Following incubation, the culture was centrifuged, the decantate discarded, and cells resuspended in buffered water. Optical density of the cell suspension was measured with a Coleman Jr. spectrophotometer. In general, the viable cell count ranged from 1 to 3×10^9 cells per ml by the pour plate method.

Carrier system

Two systems were used in these studies. The closed can system (19th Quarterly Report) was used for the dry heat inactivation studies, and a three-neck (500-ml) distillation flask was used for the wet heat studies, which are discussed in this report.

Dry heat study

In the closed can system, stainless steel cups placed in a petri dish were inoculated with a microburette in 0.01 ml amounts to give about 1×10^7 cells per cup. The inoculated cups were dried at room temperature in a dessicator jar containing P_2O_5. The dried cups were then transferred to an equilibration hood set at 1% RH at room temperature.

Lids and cans containing four shelves with uninoculated stainless steel cups were dried in a 50-C oven for 30 min. Following the drying process, these items were removed from the oven and placed in the equilibration hood overnight. The top three shelves were removed from each can and the inoculated cups were placed at the outer periphery of the bottom shelf. The remaining shelves and cups were replaced in the can. An appropriate amount of water was added in the center cups of the second shelf, and the can was sealed immediately with an electric can sealer.

After the cans were sealed, they were removed from the hood and the seams of each can were soldered. Heat resistance was measured by a constant temperature oil bath set at 60 C. A comeup time of 11.6 min was determined prior to these runs. At predetermined time intervals, cans were removed from the water bath and immediately cooled in a 4-C circulating water bath. After they were cooled, the cans were opened aseptically with an automatic can opener. Sterile microbeads were added to each sample cup, placed in 10 ml of sterile peptone water, and sonified for 10 min. Staphylococcus assays were made by the pour plate technique, using fortified TSA. The plates were incubated for 2 days at 35 C.

Wet-heat study

A 500-ml, three-neck distillation flask was immersed in a 60-C water bath so that the level of water in the bath was 2.5 cm above the level of the test medium.

The test inoculum was added in the 60-C test medium (buffered water) to give a concentration of about 10^6 Staphylococcus per ml. The test solution was agitated througout the heating experiment with a magnetic stirrer. A thermometer was mounted in one of the side-arms to monitor the solution temperature; the other arm was used for the introduction of the inoculum and the withdrawal of samples during the experiment. Five-ml samples were withdrawn at appropriate time intervals and introduced in 16 x 125 mm screw-cap tubes immersed in ice water. Assay technique used was by the pour plate technique in fortified TSA.

RESULTS AND DISCUSSION

The corrected results at 60 C are shown in Table 1. D values in minutes are shown for 13 runs. One of these experiments was performed by inserting cells into a three-neck flask (containing 60 C buffered water) that was submerged in a 60-C water bath. The D value for this run was 1.0 min. The resistance of the cells increased as the % RH was lowered. There is general agreement with a_w results that even though the substances used (NaCl, glycerol, and sucrose) tend to have a deleterious effect below 0.85, the a_w is not lowered below this level. In the level between 0.90 and 1.00, sucrose has an enhancement effect on the resistance of some organisms.

Values of thermal resistance observed in protective substrates have been reported. For example, Angelotti (1) found that strain 196E had a D value of 59 min in custard and 47 min in chicken á la king. This

study indicates that varying % RH can further increase the levels of the D value. In general, the most resistant point of % RH was between 1 and 10. These D values for % RH decrease below 1.

Figure 1 is a plot of the data in Table 1 and shows two peaks, the second occurring at 90%. Some a_w results (2) indicate a peak of 90 to 100%. This small aberration is not significant compared to the resistance in the 5% RH region.

The data are fitted to a cubic equation (Figure 1), and the peak around 4% is similar to results obtained from spores (3). Also, the inflection point at 50% is similar to the resistance observed in spore investigations (3). These similarities may indicate a common mechanism of inactivation.

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Table 1. Thermal Resistance of Staphylococcus aureus strain 472 with % RH at 60 C

Run No.	Initial count N_0	Time to reach 10 min	% RH	Est. of D value (min)
Flask	1.3×10^7	6	100	1.0
11	2×10^6	58.4	100	12.7
6B	1.2×10^6	88.4	95	19.4
7	4×10^6	108.4	90	27.7
7A	1×10^6	108.4	85	24.2
6A	1.2×10^6	88.4	80	17.9
9	1×10^6	68.4	50	15.3
9A	6.3×10^5	348	40	83.4
17	6.3×10^5	a	30	191.5
17C	3×10^5	a	10.1	440.5
17B	3×10^5	a	3.7	595.3
17A	3×10^5	a	0.19	476.2
	4×10^5	a	0.19	395.3

a - About one $\log_{10}$ reduction.

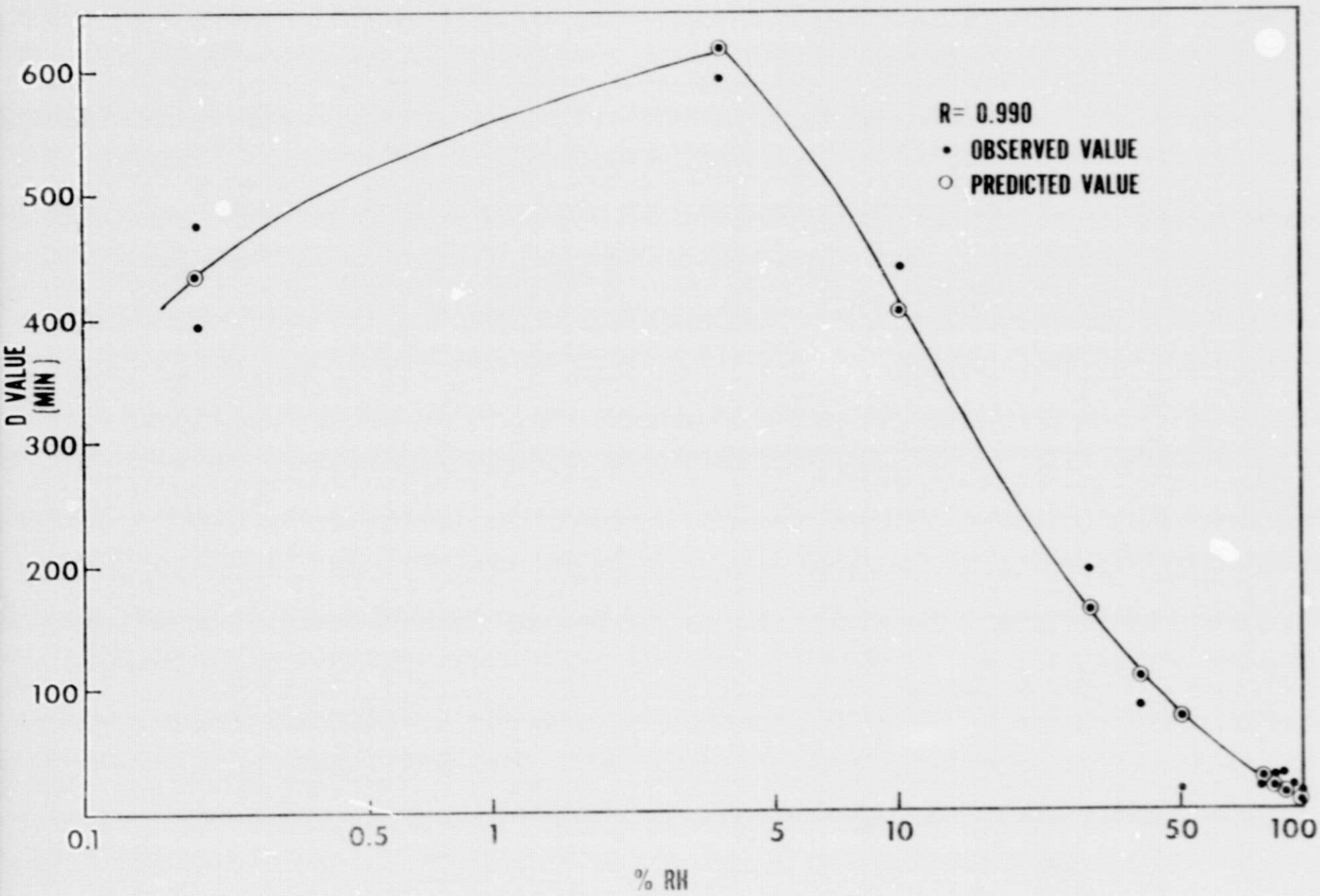

FIGURE 1. RELATION OF THERMAL RESISTANCE (D) TO MOISTURE CONTENT (% RH) OF A CLOSED SYSTEM.